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VISCOSITY CHANGES IN THE CYTOPLASM DURING THE FIRST DEVELOPMENTAL STAGES IN THE FROG EGG

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VISCOSITY CHANGES IN THE CYTOPLASM DURING THE FIRST DEVELOPMENTAL STAGES IN THE FROG EGG

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ABSTRACT. The author studied changes in the viscosity of *Rana fusca* eggs (unfertilized eggs kept in fresh water, unfertilized eggs left in the dissected oviduct, and unfertilized eggs kept in Göthlin's solution for various lengths of time; and in fertilized eggs at various times following insemination). Viscosity was determined by the degree to which 3 min of centrifugation (radius, 14 cm; rate, 2500 rpm) separated pigment and yolk particles from clear cytoplasm in a given specimen. It was found that there is a great difference in viscosity between unfertilized eggs kept in fresh water and fertilized eggs 2 hrs, 3 hrs, and 3 hrs 30 min after insemination. The viscosity decrease in unfertilized eggs kept in fresh water is attributed to incipient cytolysis due to water absorption. The viscosity of the fertilized eggs drops sharply just before each cleavage and increases again afterwards, to remain high until the next cleavage is ready to occur. These periodic fluctuations in viscosity, related to the rhythm of cell division, coincide with periods of maximum and minimum susceptibility to damage by KCN, hypoxia, hypothermia, and hyperthermia.

Introduction

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In the spring of 1920 and 1921 I began centrifugation experiments on the eggs of *Rana fusca*, with the intention of eventually studying fertilization-induced viscosity changes in the cytoplasm of the amphibian egg.

From the studies of O. Hertwig, Wetzel, Morgan and Gurwitsch, among others, we know that when the amphibian egg is subjected to a sufficiently large centrifugal force, the various cell substances separate into layers according to their densities. During centrifugation, the egg naturally presents its heavy, vegetative end toward the periphery. The heavier components of the egg cell thus collect in the vegetative half of the egg during centrifugation, while the pigment granules from the animal pole are thrown toward the equatorial zone of the egg. The animal calotte of the egg will thus consist of protoplasm more or less free of pigment and yolk granules. This redistribution of cell matter in layers can even be seen externally as a more or less complete depigmentation at the animal pole, which becomes more complete as the centrifugal force more fully overcomes the viscosity of the protoplasm. After this grey-white animal part comes a dark ring of pigment, and then a layer of clear deutoplasm.

Thus the degree of pigment displacement and the number of yolk granules

*Numbers in the margin indicate pagination in the foreign text.

remaining in the plasm in various centrifugation experiments at a given rotational velocity, provides a good relative index of the protoplasm viscosity.

Intending to study, among other things, the structure of protoplasm, Gurwitsch [1904, 1908] centrifuged fertilized *Triton* eggs for 4-5 hrs at a low rotational velocity and fertilized frog eggs for 15 min "at top speed". From /611 the fact that the eggs can develop even though the yolk elements have been forcibly separated from the protoplasm, Gurwitsch concludes that the protoplasm cannot have a solid consistency, but must be composed of fluid substances. The following is quoted from the results and conclusions of Gurwitsch:

"Investigation of the individual layers showed the well-known, very regular layering of plasm with large bubbles at the animal surface, and a thicker yolk-free layer at the bottom, which was sharply differentiated from the yolk.. .. The interfaces between the foamy and dense plasms form a pronounced meniscus within each blastomere, and the walls of this meniscus rise very steeply on the medial wall of the blastomeres. The plasma-yolk interface participates in an even more striking manner in the formation of the meniscus... Nevertheless, the meniscus appears only during the formation of the cleavage groove, before the short tongues of the yolk plaques have reached the base of the groove. In cases where the groove has already cut deep into the yolk mass, the meniscus is no longer found; the boundary layers become flat."

From the facts which he presented, Gurwitsch draws the following conclusions concerning the aggregation state of the protoplasm: "The plasm layer below the meniscus boundary has a certain degree of tensile strength, and therefore falls in the range of the solid phase... Prior to the appearance of the cell reticulum and groove formation, the density of the corresponding strip of plasm increases considerably, a circumstance which has often been assumed and for very good reasons, but which could not previously be strictly proven."

In her experiments, Mme. Konopacka [1908] attempted to determine the effect of centrifugation at an early stage of egg development on the direction of its further development. She made some incidental observations which are of special interest for us.

Her studies were carried out in the spring of 1906-1907. Her study material consisted of artificially fertilized eggs of *Rana fusca*, which were hand-centrifuged at 1500-1600 rpm. The results of her experiments, which must be taken into consideration, will be given. However, I would first like to remind the reader that it is known from the observations of O. Hertwig and Roux (and also from a check of their results made by Mme. Konopacka) that in *Rana fusca* the spermatic filament penetrates the ovum sheath into the ovum 1 hr after insemination. Within another 30 min, the head of the spermatozoon has finished /612 its wandering through the plasm, and 2 hrs after insemination, the union of the male and female nuclei is complete. At 2 hr 45 min, the nucleus is undergoing mitosis and 15 min later, the first cleavage groove appears.

Experiment I. Centrifugation of Unfertilized Eggs. The eggs were centrifuged at a rate of 1500 rpm. Sections of the egg revealed that the structure of the egg had undergone no substantial changes even after centrifugation for 30 min.

Experiment II. 2. A. *Centrifugation begun 15 min after insemination.* As in the following experiment, the eggs were centrifuged at a rate of 1600 rpm. The effect of centrifugation at this stage took the form of extensive changes in the egg structure. During centrifugation, the egg underwent flattening at the animal pole. The separation of the egg substances into three layers began after 10 min of rotation, but was not very pronounced. Clear layering was much more evident after 15 to 20 min.

Experiment II. 2. B. *Centrifugation begun 1.5 and 2 hrs after insemination.* Centrifugation failed to flatten the eggs at the animal pole. Even after only 5 min of rotation, separation into three layers was externally visible, and after 10 min of rotation the layering was even clearer.

Experiment II. 2. C. *Centrifugation begun 2 hr 45 min after insemination.* The eggs behaved in the same manner as those centrifuged during nuclear conjugation (series B), differing from the latter only in the fact that flattening during centrifugation did occur.

Experiment III. 2. *Eggs centrifuged immediately after appearance of the first groove.* From the results of this experiment it was found that the eggs were flattened at the animal pole and exhibited the same changes in the plasm structure as eggs centrifuged before the appearance of the first furrow. The layering of the substances was evident after only 10 min of rotation, just as in the eggs centrifuged 15 min after insemination.

The resistance of the egg to the effects of centrifugal force was designated by Mme. Konopacka, following a suggestion of Driesch, as the rigidity of the egg structure. According to the observations of Mme. Konopacka, this rigidity fluctuates even in early stages of development, the eggs having the least rigidity from the onset of zygote conjugation to the completion of the first mitotic groove.

The experiments discussed above, however, possess defects which are probably due to the fact that Mme. Konopacka's experiments were not made with the direct intent of studying changes in state of the protoplasm during fertilization, but rather the effect of centrifugation on the later development of the eggs.

The unfertilized eggs used in Experiment I cannot be directly compared /613
with the other fertilized eggs in the other experiments for several reasons. Firstly, the eggs were not centrifuged at the same rate, which in itself probably destroys comparability with the other experiments. In addition, this series of experiments was carried out toward the end of the sexual period, which means that the eggs were overripe. Egg mortality was considerable in the two experiments, 44% and 47%, which may indicate that the eggs were overripe. Finally, the orientation of the eggs during centrifugation is uncertain, since they were not centrifuged in a liquid, but in intact oviducts. Under these conditions the eggs could only turn slightly, being under constraint, as Hertwig and Wetzel have shown*.

*Mme. Konopacka herself states that these results "cannot make any claim to completeness".

In addition, I have a further objection to the procedure used in these experiments; apparently Mme. Konopacka compared eggs from different frogs in the various experiments. According to my own experience, if eggs from different females but at the same stage of development are simultaneously centrifuged, it will often happen that different portions of the egg are not affected to the same extent by the centrifugation. If fertilized and unfertilized centrifuged eggs from different females are compared with each other, the results are often so different that no definite conclusions can be drawn.

Although Mme. Konopacka did not succeed in definitely determining the relationship between the rigidity of cell substances in fertilized and unfertilized eggs, it is nontheless clearly evident from her experiments that changes in cytoplasm rigidity are closely related to the fertilization process and the formation of cleavage grooves. Mme. Konopacka did not go into the question of whether other factors, such as first of all water absorption by the egg, did not play a certain role.

Brachet [1906] made some relevant observations in his experiments on the regulation ability of punctured eggs.

Unfertilized *Rana fusca* eggs were placed on a glass slide and slightly punctured with a hot needle. Of more than 200 eggs treated in this manner, the great majority died without showing a trace of groove formation. A small number showed 2, 3 or 4 superficial and atypical grooves, and only one egg /614 reached the morula stage.

In eggs from a single female punctured 15 or 30 min after insemination, the mortality rate was also quite high, but nevertheless numerous eggs developed into normal larvas.

Up to 45 min after insemination, puncture did not impair the later development of the eggs: up to this moment, the eggs showed complete regulation ability. Bracket also believes that eggs can be assumed to possess complete regulation ability up to 1 hr after insemination. In contrast, from 1 hr after insemination on, the eggs showed an incomplete regulation ability. To be sure, the eggs continued to develop, but always abnormally. At 2 hrs, 2 hrs 15 min, and 2 hrs 30 min after insemination, puncture caused a very high mortality rate. Eggs punctured even later, just before cell division, once more showed full regulation ability, as Roux and Moszkowski also showed.

Brachet says of his results:

"It is clear from the experiments whose results I have just described that *Rana fusca* eggs have full regulatory capacity up to 1 hr after impregnation by the sperm.... But at 1 hr after fertilization, from the moment when the spermatozoon penetrates the ovum and before conjugation of the two pronuclei, the results of puncture become quite different.... It is, in fact, precisely at the moment when the ovum begins to react to the action of the spermatozoon, at the moment when the latter penetrates to the interior of the ovum leaving behind a trail of pigment, that the regulatory capacity of the ovum becomes incomplete, disappearing completely a few minutes later... In fact, at 1 hr 15

min, 1 hr 30 min, and 2 hrs after impregnation by the sperm, successful puncture experiments show the ovum to have become incapable of expelling the damaged matter."

"It is certain that an unfertilized egg, whose gelatinous matter is not yet saturated with water, is in a physical environment very different from that of an egg which has already been inundated in the spermatic fluid. It may be concluded that the effect of puncture is more violent, and that the egg is more easily killed... It seems to me likely that some factor other than that of the physical environment is involved, this factor residing without doubt in the decreased solidity of ovular protoplasm before fertilization."

"In addition, it will be remembered that the virgin egg is softer and less turgescent on puncture than the fertilized egg."

Heilbrunn [1915, 1916] found that all substances which activate the unfertilized egg of the sea urchin also cause a marked increase in the viscosity of the cytoplasm. He also found a similar increase in viscosity after normal fertilization of the egg. From these facts he concluded that it is "solidification" or jelling of the protoplasm which gives the impetus to mitosis. Among other proofs he adduces the fact that if the protoplasm is artificially kept in a liquid state,no mitotic formations appear and the egg remains ungrooved. As an index of viscosity, Heilbrunn (who was working with the pigmented eggs of *Arbacia punctulata*) used the rate at which the pigment granules moved through the egg plasm during centrifugation. With the plasm in a liquid state, a certain centrifugation rate caused all the pigment granules to gather in one half of the egg, the other half forming a clearly defined "hyaline zone". As viscosity increased, the hyaline zone became smaller, until no visible shift in pigment distribution occurred at the same or even higher rotation rates. /615

In eggs centrifuged at various times after insemination, Heilbrunn found that viscosity continued to increase between insemination and the first cleavage groove in the sea urchin egg, reaching a maximum just before the appearance of the amphiaster. Thereafter viscosity decreased to approximately its original value, and then increased once more before the appearance of the second amphiaster. In his most recent paper, Heilbrunn [1921] confirms these results for the egg of *Cumingia tellinoides* and also gives a few results for the *Nereis* egg.

In 1918, during centrifugation experiments on frog eggs in this institute, Dr. J. Runnström observed that fertilized and unfertilized eggs are not equally affected by centrifugation. It was this observation which provided the stimulus for the present investigation.

Experimental Setup

In my experiments, I used artificially fertilized eggs of *Rana fusca*. To eliminate any pathological eggs, centrifugation experiments were never carried out if at least 50% of the fertilized eggs did not show cleavage. In experiments on unfertilized eggs, the results were taken into consideration only when

at least 80% of fertilized eggs from the same female showed cleavage in later development.

In the various experiments, conducted simultaneously, only eggs from a /616
single female were used. Part of the eggs were suspended for the appropriate period of time in fresh water and part of them in the physiological salt solution isotonic with frog serum suggested by Göthlin [1899]*.

I believe the assumption is justified that eggs live in Göthlin's solution under approximately the same conditions as in the oviduct of the frog, and that eggs stored in Göthlin's solution for a certain time, which must not be excessive; can be considered equivalent to eggs taken directly from the oviduct. This assumption is based on the following: eggs stored in fresh water are known to swell due to water absorption. This is even more true of the gelatin capsules, which cling together in large clumps as soon as they come in contact with water. By contrast, eggs placed in Göthlin's solution did not swell visibly, and although the gelatin capsules did swell a little, they did not swell nearly as much in fresh-water**. Also, Göthlin eggs did not clump together like fresh-water eggs. As will be shown below, frog eggs in Göthlin's solution can be fertilized for at least 3 hrs after the female has been killed. The fact that I did not succeed in fertilizing Göthlin eggs stored for a longer time is probably due less to changes in the interior of the egg, than to the fact that the gelatin capsules had actually swollen slightly after this length of time. Here I should like to note that frog eggs in fresh water very quickly lose the ability to be fertilized, after only a few minutes. Again, this seems more likely due to changes in the gelatin capsules than to changes in the interior of the egg.

Simultaneous centrifugation of eggs, some of which were stored for a certain length of time in Göthlin's solution and others in an intact oviduct (with a small amount of Göthlin's solution added to facilitate orientation of the eggs) was found to affect the eggs to the same extent.

This provides a sufficient basis for assuming that Göthlin's solution does not basically change the state of the cytoplasm.

The use of Göthlin's solution permitted simultaneous centrifugation of both fertilized and unfertilized eggs from the same female. The unavoidable error introduced by comparison of eggs from different females, which are therefore in various stages of maturity, is thus eliminated. Göthlin's solution has the further advantage of permitting determination of whether the different behavior of fertilized and unfertilized eggs during centrifugation is related to the absorption of fresh-water by the egg.

The centrifugations were carried out on an electric-powered centrifuge at a constant rotation rate of about 2500 rpm. The radius of this centrifuge was 14 cm, and the tubes in which the eggs were placed during centrifugation

*Göthlin's solution has the following composition: 0.65% NaCl, 0.01% KCl, 0.0065% $CaCl_2$, 0.1% $NaHCO_3$.

**In the following, "fresh-water eggs" and "Göthlin eggs" will designate eggs from a single female stored for equal times in one or the other fluids.

had a interior diameter of 3 cm. Each sample was weighed with great care to ensure that the egg batches to be compared would have the same weight. In addition, a relatively small number of eggs was used in each experiment (20 to 25 eggs), to minimize variation in the distance of the eggs from the center of rotation.

Centrifugation Experiments to Determine the Effect of Fertilization on Cytoplasm Viscosity

Experiment I. 1. Centrifugation was done 1 hr after insemination with a) fertilized eggs; b) fresh-water eggs; c) Göthlin eggs. The duration of centrifugation was 3 min, as in the following experiments.

Results: a) and b) showed slight depigmentization at the animal end, to about an equal degree.

c) The great majority of these eggs appeared normal, though very slight depigmentization could be discerned in individual eggs.

Experiment I. 2. Eggs from a single female centrifuged 2 hrs after insemination.

Results: a) and b). Depigmentization was somewhat more pronounced than in the previous experiment, the eggs in a) seeming in general more strongly affected by centrifugation than those in b).

c) See *Experiment I.* 1.c.

Experiment I. 3. Centrifugation 3 hrs after insemination.

Results: a) The eggs showed a notable shift in pigmentization. At the animal pole, a clearly defined field with many discontinuities formed, which was about the size of the normal yolk field at the vegetative pole.

b) Depigmentization was a little more masked than in *Experiment I.* 2.

c) No depigmentization visible.

Experiment I. 4. Eggs centrifuged just before the appearance of the first cleavage groove. During or shortly after centrifugation, the first groove appeared (about 3 hrs 30 min after insemination).

Results as in the preceding experiment.

Experiment I. 5. Eggs centrifuged immediately after appearance of the first groove. /618

Results: a) The pigment shift was approximately as great as in the preceding experiment. The clear field at the pole was sharply delineated from the ring of pigment, but the center of the animal pole was not yet completely

depigmentized.

b) and c) See preceding experiment.

Experiment I. 6. Centrifugation 20 min after the appearance of the first groove.

Results: a) Pigment shift was significantly less than in the last three experiments.

b) The fresh-water eggs appeared to be affected in approximately the same degree as the fertilized eggs. However, depigmentization was not complete, since the pigment appeared as light stripes and spots surrounding the center of the animal pole in the form of a horseshoe or a ring.

c) See *Experiment I.* 1.c.

Experiment I. 7. Shortly before the second division, centrifugation was repeated (4 hrs 10 min after insemination).

Results: a) The eggs appeared to be affected by centrifugation in about the same degree as in *Experiments I.* 3.a. and *I.* 4.a.

b) The eggs had the same appearance as in the preceding experiment, but were affected considerably less than the fertilized eggs in this experiment.

c) See *Experiment I.* 1.c.

Experiment I. 8. The eggs were centrifuged immediately after appearance of the second groove (4 hrs 20 min after insemination).

Results: a) Depigmentization was about the same as or a little bit more pronounced than in *Experiment I.* 5.a.

b) See the preceding experiment.

c) See *Experiment I.* 1.c.

Experiment I. 9. Eggs centrifuged 30 min after the appearance of the second groove.

Results: a) See *Experiment I.* 6.a.

b) The eggs were generally affected by centrifugation significantly more than the fertilized eggs. In comparison to *Experiment I.* 8.b., the eggs were changed somewhat more by acceleration.

c) See *Experiment I.* 1.c.

Experiment I. 10. Centrifugation was repeated 10 min before the third division.

Results: a) The strikingly severe depigmentization was most closely comparable to the results of *Experiments I.* 3.a. and *I.* 7.a.

b) The great majority of eggs showed a smaller degree of depigmentization than the fertilized eggs; however, in individual eggs, depigmentization was almost as great as in the fertilized eggs.

c) See *Experiment I.* 1.c.

The percentage of eggs in this experimental series which developed furrows was 92%, and these eggs developed into larvae quite normally. Various experiments, particularly those with furrowed eggs, were repeated many times, always with the same results.

To confirm my assumption that eggs in Göthlin's solution enjoy environmental conditions similar to those in the oviduct, I made the following experiments: /619

Experiment II. Eggs from a single female, part stored 2 hrs in Göthlin's solution and part in an intact oviduct, were centrifuged simultaneously for 5 min. A little Göthlin's solution was added to the eggs in the oviduct to make pole orientation possible.

It was found that both the oviduct eggs and the Göthlin eggs showed slight depigmentization, which was exactly the same for both kinds of eggs. The experiment was repeated with the same results 3 hrs 30 min and 4 hrs 30 min after the female was killed.

Experiment III. Göthlin eggs were centrifuged for 3 min, 24 hrs after killing the female.

Results: Eggs were not noticeably affected by centrifugation.

Experiment IV. Göthlin eggs were transferred to fresh water 3 hrs after killing the female and fertilized there. (Many attempts to fertilize eggs directly in Göthlin's solution were unsuccessful).

Results: Out of six such experiments on about 50 eggs each, three were unsuccessful. In a fourth experiment, cleavage occurred normally, but the majority of eggs died in the blastula stage and only a few developed up to the beginning of gastrulation. Thus from two batches of eggs, I obtained 17 and 19 normal larvae respectively, a result which must be considered very good, considering that insemination was done in fresh water.

Numerous attempts to bring the eggs to the cleavage stage after 3 hrs 30 min in Göthlin's solution were always unsuccessful.

The impossibility of fertilizing the eggs directly in Gothlin's solution is because in Göthlin's solution, as in the testicles, the spermatozoa possess no motility. Observation of spermatozoa kept for 24 hours in Göthlin's solution showed that they had no motility, as mentioned above. However, if the

osmotic pressure was lowered by adding water, the sperms immediately began to perform their characteristic movements. The fertilization capacity of these sperm remained unchanged. Similar observations were subsequently made on *Bufo calamita*, *Triton cristatus*, and *Osmerus eperlanus* (using an isotonic Ringer solution). I do not know whether similar observations have been made before; I am presenting mine in any case, since experiments on the mechanics of development involving artificial insemination often require the investigator to work with small amounts of material, and it would be of great help if sperm could be stored from day to day.

Observations of Sections /620

The eggs were fixed in a 2% formalin solution heated to about 80° immediately after removal from the centrifuge, using the method suggested by O. Schulze. I believe that I can assume that this simple and advantageous method is completely sufficient in this case, which only requires fixation of the position of the various cell substances.

Investigation of the sections fully confirmed the results obtained by examination of the intact egg. It was found that Göthlin eggs, whether kept in Göthlin's solution for only 1 hr or up to 25 hrs, were affected only insignificantly or not at all by centrifugal acceleration. In fresh-water eggs, the effect of centrifugal force increased with longer storage time. The plasm part of the centrifuged egg, which took up almost a third of the volume of the egg in *Experiments I.* 9.b. and *I.* 10.b, was never sharply separated from the deutoplasm portion.

Momentarily disregarding *Experiments I.* 6.a. and *I.* 9.a., the plasm part of the fertilized eggs was completely freed of pigment and yolk elements and was clearly divided from the yolk. Division of the protoplasm into two layers, observed by Gurwitsch, was seen in my experiments as a darker staining of the plasm layer next to the animal poles with Mallory's aniline blue. Part of the pigment accumulated at the interface between the protoplasm and the deutoplasm, and /621 part had been driven into the deutoplasm in clumps and stripes.

Figure 1 shows an egg centrifuged 3 hrs after insemination.

In the section reproduced in Figure 2, where the first cleavage groove has just appeared, the meniscus formation mentioned by Gurwitsch is visible. Between the pigmented lines where the first cleavage will cut through later, the plasm is richly sown with pigment granules and yolk platelets.

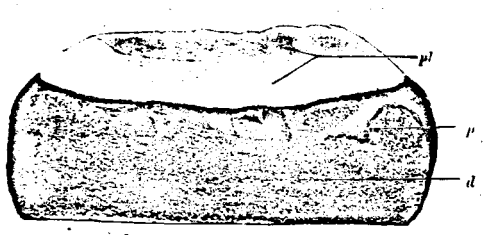

Figure 1. *pl* = layer of Pure Plasm; *p* = Pigment; *d* = Deutoplasm.

Figure 3, where the groove has cut somewhat deeper into the inside of the egg, also shows this type of meniscus formation.

Figure 4 shows an egg in which the first cleavage groove is complete. A small portion

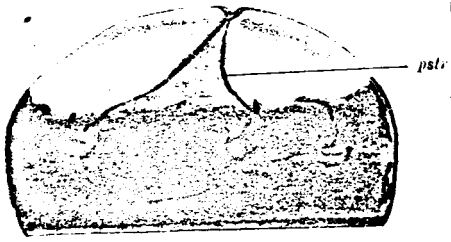

Figure 2. *pstr* = Pigment Stripe.

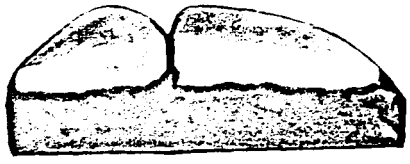

Figure 3.

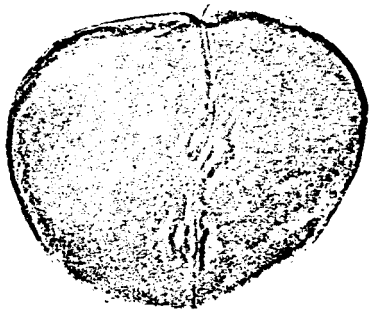

Figure 4.

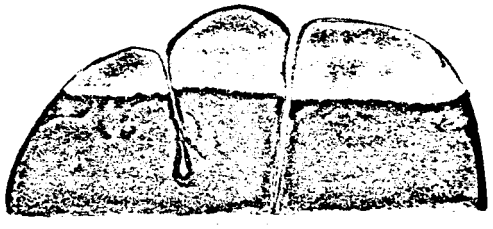

Figure 5.

of plasm can still be seen at the animal pole, but this has not been freed of pigment and yolk elements. In this section, which was made through the center of the egg, the groove is marked by accumulations of pigment, which does not occur when the section is made some distance from the longitudinal axis. In this case, a meniscus was not formed.

In Figure 5, where the second groove has cut into the yolk material, the plasm-yolk boundary surface is also flat. As in the sections of the single-celled stage, which show a similar groove formation, the second groove is closely surrounded by pigment granules. In contrast, this is not true of the first groove visible in the same section.

The distribution of pigment along the most recently formed furrow was already mentioned by Nussbaum [1893] for our experimental subject. This observation has recently been studied, especially by Spek [1918].

Discussion of Results /622

My experiments show that during the first developmental stage of the egg of *Rana fusca*, the viscosity of the cytoplasm changes significantly. My intention was primarily to determine whether fertilization causes a change in the physical state of the cytoplasm which could be demonstrated as a viscosity change. The most outstanding result of my experiment is actually the great difference in viscosity between unfertilized fresh-water eggs and fertilized eggs 2 hrs or even 3 hrs or 3 hrs 30 min after insemination. Thus, viscosity decreases sharply a certain length of time after insemination, and this decrease cannot be attributed to water absorption by the cytoplasm. The water absorption is initially equal in fertilized and unfertilized eggs. Comparison with Göthlin eggs actually shows that in unfertilized fresh-water eggs, a small decrease in viscosity, obviously due to water absorption, did appear. However, at a certain point in time, the unfertilized eggs are greatly affected by centrifugal force, and definite signs of incipient cytolysis appear. The unfertilized fresh-water eggs differed very greatly with respect to the onset point of this loss of viscosity due to incipient cytolysis, as for example in *Experiment I.*

10.b. presented above. The decrease in viscosity does not occur directly after fertilization (1 hr after insemination). It only becomes noticeable an hour after fertilization and reaches a maximum shortly before the first cell division. The change in the regulation capacity of the egg plasm, which Brachet found after insemination, thus cannot depend on a change in viscosity. Possibly other changes must be considered -- for example, changes in surface tension.

These experiments are very similar to those of Heilbrunn [1920-1921], who found that after fertilization there is a period of higher viscosity followed by a period of lower viscosity. However, in Heilbrunn's experiments, eggs in the first period after fertilization as well as unfertilized eggs are characterized by relatively low viscosity. Only during the first spindle formation, as was mentioned above, does the viscosity increase. I did not succeed in demonstrating any correlation between viscosity increase and the state of the unfertilized eggs after fertilization. It is therefore quite obvious that the large viscosity decrease is reversible. After formation of the second groove, /623 the viscosity once more increases and reaches the same level as in the unfertilized eggs, as shown by *Experiment I.* 6. and others. But before the second cleavage, viscosity drops to the same low value as before the first cleavage (*Experiments I.* 7, etc.). Then after formation of the second cleavage groove, viscosity once more increases, as shown by *Experiment I.* 9 and others. From this experiment we seem to find that the viscosity in the latter case becomes greater than that of fresh-water eggs. Nevertheless, the circumstances discussed earlier must be considered. Unfertilized eggs kept for almost 5 hrs in fresh water are changed by water absorption. Thus we have not found out whether the fertilized eggs actually become more viscous than unaltered, unfertilized fresh-water eggs. It does not seem very likely to me that this is the case. In any case, they do not attain the same viscosity as Göthlin eggs. This pronounced viscosity decrease appears once more before the third cleavage.

The results reported here, make it clear that periodic variations in the cytoplasm viscosity occur in the frog egg. Evidently, this periodicity is related to the rhythm of cell division. My results thus agree in principle with those of Heilbrunn. As I mentioned earlier, he considered the increase in viscosity to mark the beginning of gel formation, the formation of the spindle. It is hardly possible to find any other explanation for the behavior of the *Arbacia* and the *Cumingia* eggs. We must also remember that the periodicity in the viscosity of the protoplasm found by Heilbrunn agrees well with the periodic variations in resistance to various external factors first observed by Lyon [1902, 1904]. If we consider the results of Heilbrunn and Lyon together, the viscosity maximum coincides with maximum susceptibility to injury by KCN, hypoxia, and cold, and with minimum susceptibility to damage by heat.

Thus, periodic changes in the physical state of the cytoplasm must also take place in the frog egg. I am inclined to relate this fact to changes in the binding of water by the plasm colloids, but cannot be more specific at this point. It is difficult to believe that the marked drop in viscosity occurring prior to cell cleavage can be due solely to "spindle coagulation" [Heilbrunn, 1921]*. I tend rather to believe that during the viscosity decrease, there /624

*See page 13 for footnote.

occurs a change in the colloids due to other causes, a change which is probably significant for the penetration of the grooves. The effect of surface tension during cleavage is facilitated or even made possible by the more fluid state of the cytoplasm. It is very interesting for the physiology of fertilization to note that unfertilized eggs only attain the low viscosity found in certain phases of the fertilized eggs with the onset of cytolysis in fresh water.

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*For one thing, the decrease is considerable from the initial value (before spindle formation). Furthermore, the spindles and spheres in the frog egg include a relatively small proportion of the cytoplasm, which Heilbrunn states is also true of the *Nereis* egg.

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